[0544] FIGS. **1** and **2** represent a XRPD and MDSC scan, respectively, of a salt prepared according to Preparation A.

Preparation B

[0545] Dissolve 4.23 g of N-(3-Aminopropyl)-N—[(R)-1-3-benzyl-7-chloro-4-oxo-4H-chromen-2-yl)-2-methyl-propyl]-4-methyl-benzamide in 5 volumes (21 ml) of THF. Add 10 volumes (42 ml) of TBME (tert-butyl methyl ether), then 1.1 eq of 12N HCl (750 μl) in one portion. Seed with HCl salt such as prepared in Preparation A (e.g. in 3 additions of about 10 mg each staggered over about 10 minutes). Stir the mixture overnight, e.g. for about 12-24 hours. Filter the mixture, wash the solids with TBME, and dry the solids in a vacuum oven at room temperature overnight, e.g. for about 12-24 hours, to yield about 4.3 g of HCl salt.

[0546] FIG. **3** represents an XRPD scan of a salt prepared according to Preparation B.

[0547] The present invention includes crystalline N-(3-Aminopropyl)-N—[(R)-1-3-benzyl-7-chloro-4-oxo-4H-chromen-2-yl)-2-methyl-propyl]-4-methyl-benzamide hydrochloride salt having characteristic X-ray diffraction peaks at about 4.8, 9.7, 14.7, 17.9, 18.3, 20.1, 20.9, 22.5, 23.2, 23.8, 26.1 and 26.9 degrees 2 theta.

Example 22

Inhibition of Cellular Viability in Tumor Cell Lines Treated with KSP Inhibitors,r

Materials and Solutions:

[0548] Cells: SKOV3, Ovarian Cancer (human).

[0549] Media: Phenol Red Free RPMI+5% Fetal Bovine Serum+2 mM L-glutamine.

[0550] Colorimetric Agent for Determining Cell Viability: Promega MTS tetrazolium compound.

[0551] Control Compound for max cell kill: Topotecan, 1 μM.

[0552] Procedure: Day 1- Cell Plating:

[0553] Adherent SKOV3 cells are washed with 10 mLs of PBS followed by the addition of 2 mLs of 0.25% trypsin and incubation for 5 minutes at 37° C. The cells are rinsed from the flask using 8 mL of media (phenol red-free RPMI+5% FBS) and transferred to fresh flask. Cell concentration is determined using a Coulter counter and the appropriate volume of cells to achieve 1000 cells/100 μL is calculated. 100 μL of media cell suspension (adjusted to 1000 cells/100 μL) is added to all wells of 96-well plates, followed by incubation for 18 to 24 hours at 37° C. 100% humidity, and 5% CO_2, allowing the cells to adhere to the plates.

Procedure: Day 2- Compound Addition:

[0554] To one column of the wells of an autoclaved assay block are added an initial 2.5 μL of test compound(s) at 400× the highest desired concentration. 1.25 μL of 400× (400 μM) Topotecan is added to other wells (optical density's from these wells are used to subtract out for background absorbance of dead cells and vehicle). 500 μL of media without DMSO are added to the wells containing test compound, and 250 μL to the Topotecan wells. 250 μL of media+0.5% DMSO is added to all remaining wells, into which the test compound (s) are serially diluted. By row, compound-containing media is replica plated (in duplicate) from the assay block to the corresponding cell plates. The cell plates are incubated for 72 hours at 37° C., 100% humidity, and 5% CO_2.

Procedure: Day 4-MTS Addition and OD Reading:

[0555] The plates are removed from the incubator and 40 μl MTS/PMS is added to each well. Plates are then incubated for 120 minutes at 37° C., 100% humidity, 5% CO_2, followed by reading the ODs at 490 nm after a 5 second shaking cycle in a ninety-six well spectrophotometer.

Data Analysis

[0556] The normalized % of control (absorbance- background) is calculated and an XLfit is used to generate a dose-response curve from which the concentration of compound required to inhibit viability by 50% is determined. The compounds of the present invention show activity when tested by this method as described above.

Example 23

Enantiomer Separation

[0557] An enriched 3:1 R:S mixture of chromenone enantiomers was separated into its pure enantiomers by chiral chromatography with the following conditions: Column—Chiralpak AD, 250×4.6 mm (Diacel Inc.). Sample—22.5 mg/ml in 1:1 i-PrOH:hexanes. Conditions—40 min at isocratic 50% i-PrOH in Hexanes. (S)-enantiomer elutes at 18.35 min. (R)-enantiomer elutes at 26.87 min. The (R)-enantiomer was significantly more potent than the (S)-enantiomer of the compound of Example 2.

Example 24

Monopolar Spindle Formation Following Application of a KSP Inhibitor

[0558] Human tumor cells Skov-3 (ovarian) were plated in 96-well plates at densities of 4,000 cells per well, allowed to adhere for 24 hours, and treated with various concentrations of the chromenone compounds for 24 hours. Cells were fixed in 4% formaldehyde and stained with antitubulin antibodies (subsequently recognized using fluorescently-labeled secondary antibody) and Hoechst dye (which stains DNA).

[0559] Visual inspection revealed that the compounds caused cell cycle arrest in the prometaphase stage of mitosis. DNA was condensed and spindle formation had initiated, but arrested cells uniformly displayed monopolar spindles, indicating that there was an inhibition of spindle pole body separation. Microinjection of anti-KSP antibodies also causes mitotic arrest with arrested cells displaying monopolar spindles.

Example 25

Inhibition of Cellular Proliferation in Tumor Cell Lines Treated with KSP Inhibitors

[0560] Cells were plated in 96-well plates at densities from 1000-2500 cells/well of a 96-well plate and allowed to adhere/grow for 24 hours. They were then treated with various concentrations of drug for 48 hours. The time at which compounds are added is considered T_0. A tetrazolium-based assay using the reagent 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) (U.S. Pat. No. 5,185,450) (see Promega product catalog #G3580, CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay) was used to determine the number of viable cells at T_0 and the number of cells remaining after 48 hours